

SEQUENCE LISTING



<110> Genencor International, Inc.
Poulouse, Ayrookaran J.

<120> Multiply-Substituted Protease Variants

<130> GC717-2-PCT

<140> PCT/US03/01447

<141> 2003-01-16

<150> US 60/350,222

<151> 2002-01-16

<160> 10

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 1494

<212> DNA

<213> Bacillus amyloliquefaciens

<400> 1

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gcacgatgag	cgccgctaag	aagaaagatg	tcattttctga	aaaaggcggg	aaagtgcaaa	300
agcaattcaa	atatgtagac	gcagcttcag	ctacattaaa	cgaaaaagct	gtaaaagaat	360
tgaaaaaaga	cccagcgcgc	gcttacggtg	aagaagatca	cgtagcacat	gcgtacgcgc	420
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taaaggtagc	aggcggagcc	agcatgggtc	cttctgaaac	aaatcctttc	caagacaaca	600
actctcacgg	aactcacggt	gccggcacag	ttgcggctct	taataactca	atcgggtgat	660
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caagcacagt	gggctaccct	ggtaaatacc	cttctgtcat	tgcagtaggc	gctgttgaca	960
gcagcaacca	aagagcatct	ttctcaagcg	taggacctga	gcttgatgtc	atggcacctg	1020
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tggcatctcc	gcacgttgcc	ggagcggctg	ctttgattct	ttctaagcac	ccgaactgga	1140
caaacactca	agtcgcgagc	agtttagaaa	acaccactac	aaaacttggt	gattctttct	1200
actatggaaa	agggtgatc	aacgtacagg	cggcagctca	gtaaaacata	aaaaaccggc	1260
cttggccccg	ccggtttttt	atttttcttc	ctccgcatgt	tcaatccgct	ccataatcga	1320
cggatggctc	cctctgaaaa	ttttaacgag	aaacggcggg	ttgaccgggc	tcagtcccgct	1380
aacggccaag	tcctgaaacg	tctcaatcgc	cgcttcccg	tttccgggtca	gctcaatgcc	1440
gtaacggctc	gcggcggttt	cctgataccg	ggagacggca	ttcgtaatcg	gac	1494

<210> 2

<211> 382

<212> PRT

<213> Bacillus amyloliquefaciens

<220>
 <221> VARIANT
 <222> 163, 164
 <223> Xaa = Pro or Asn

<221> VARIANT
 <222> 168
 <223> Xaa = Asp or Asn

<221> VARIANT
 <222> 195, 196
 <223> Xaa = Ser or Ala

<221> VARIANT
 <222> 205, 206
 <223> Xaa = Asp or Ala

<221> VARIANT
 <222> 265, 266
 <223> Xaa = Ser or Thr

<221> VARIANT
 <222> 358
 <223> Xaa = Glu or Gln

<400> 2
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 Ile Phe Thr Met Ala Phe Gly Ser Thr Ser Ser Ala Gly Ala Ala Gly
 20 25 30
 Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met
 35 40 45
 Ser Thr Met Ser Ala Ala Lys Lys Lys Asp Val Ile Ser Glu Lys Gly
 50 55 60
 Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala Ala Ser Ala Thr
 65 70 75 80
 Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala
 85 90 95
 Tyr Val Glu Glu Asp His Val Ala His Ala Tyr Ala Gln Ser Val Pro
 100 105 110
 Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln Gly Tyr
 115 120 125
 Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser
 130 135 140
 Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala Ser Met Val Pro Ser
 145 150 155 160
 Glu Thr Xaa Xaa Phe Gln Asp Xaa Asn Ser His Gly Thr His Val Ala
 165 170 175
 Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala
 180 185 190
 Pro Ser Xaa Xaa Leu Tyr Ala Val Lys Val Leu Gly Xaa Xaa Gly Ser
 195 200 205
 Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn
 210 215 220
 Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly Pro Ser Gly Ser Ala
 225 230 235 240

Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala Ser Gly Val Val Val
 245 250 255
 Val Ala Ala Ala Gly Asn Glu Gly Xaa Xaa Gly Ser Ser Ser Thr Val
 260 265 270
 Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala Val Gly Ala Val Asp
 275 280 285
 Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro Glu Leu Asp
 290 295 300
 Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys
 305 310 315 320
 Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser Pro His Val Ala Gly
 325 330 335
 Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Trp Thr Asn Thr Gln
 340 345 350
 Val Arg Ser Ser Leu Xaa Asn Thr Thr Thr Lys Leu Gly Asp Ser Phe
 355 360 365
 Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala Ala Ala Gln
 370 375 380

<210> 3
 <211> 275
 <212> PRT
 <213> Bacillus amyloliquefaciens

<400> 3
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 His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
 20 25 30
 Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala
 35 40 45
 Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His
 50 55 60
 Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly
 65 70 75 80
 Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
 85 90 95
 Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
 100 105 110
 Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
 115 120 125
 Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala
 130 135 140
 Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly
 145 150 155 160
 Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala
 165 170 175
 Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val
 180 185 190
 Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
 195 200 205
 Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser
 210 215 220
 Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn
 225 230 235 240
 Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys
 245 250 255

Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
 260 265 270
 Ala Ala Gln
 275

<210> 4
 <211> 275
 <212> PRT
 <213> Bacillus subtilis

<400> 4
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 His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
 20 25 30
 Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala
 35 40 45
 Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His
 50 55 60
 Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly
 65 70 75 80
 Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
 85 90 95
 Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
 100 105 110
 Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
 115 120 125
 Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser
 130 135 140
 Ser Gly Ile Val Val Ala Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly
 145 150 155 160
 Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala
 165 170 175
 Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala
 180 185 190
 Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
 195 200 205
 Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr
 210 215 220
 Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Thr
 225 230 235 240
 Trp Thr Asn Ala Gln Val Arg Asp Arg Leu Glu Ser Thr Ala Thr Tyr
 245 250 255
 Leu Gly Asn Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
 260 265 270
 Ala Ala Gln
 275

<210> 5
 <211> 274
 <212> PRT
 <213> Bacillus licheniformis

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 Gln Ala Gln Gly Phe Lys Gly Ala Asn Val Lys Val Ala Val Leu Asp

130		135		140											
Val	Leu	Val	Val	Ala	Ala	Ser	Gly	Asn	Ser	Gly	Ala	Gly	Ser	Ile	Ser
145				150						155					160
Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val	Gly	Ala	Thr	Asp	Gln
				165						170					175
Asn	Asn	Asn	Arg	Ala	Ser	Phe	Ser	Gln	Tyr	Gly	Ala	Gly	Leu	Asp	Ile
				180						185					190
Val	Ala	Pro	Gly	Val	Asn	Val	Gln	Ser	Thr	Tyr	Pro	Gly	Ser	Thr	Tyr
		195					200					205			
Ala	Ser	Leu	Asn	Gly	Thr	Ser	Met	Ala	Thr	Pro	His	Val	Ala	Gly	Ala
		210					215				220				
Ala	Ala	Leu	Val	Lys	Gln	Lys	Asn	Pro	Ser	Trp	Ser	Asn	Val	Gln	Ile
225					230					235					240
Arg	Asn	His	Leu	Lys	Asn	Thr	Ala	Thr	Ser	Leu	Gly	Ser	Thr	Asn	Leu
				245						250					255
Tyr	Gly	Ser	Gly	Leu	Val	Asn	Ala	Glu	Ala	Ala	Thr	Arg			
			260					265							

<210> 7
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer

<400> 7
 gtgtgtggggc ccatcagtct gacgacc

27

<210> 8
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer

<400> 8
 gtgtgtggggc cctattcgga tattgag

27

<210> 9
 <211> 275
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> consensus sequence

<221> VARIANT
 <222> (1)...(275)
 <223> Xaa = Any Amino Acid

<400> 9															
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			20					25					30		

Xaa	Gly	Xaa	Xaa	Xaa	Xaa	His	Pro	Asp	Leu	Xaa	Xaa	Xaa	Gly	Gly	Ala
	35						40						45		
Ser	Xaa	Val	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Gln	Asp	Xaa	Asn	Xaa	His
	50					55					60				
Gly	Thr	His	Val	Ala	Gly	Thr	Xaa	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly
65					70					75					80
Val	Leu	Gly	Val	Ala	Pro	Ser	Ala	Xaa	Leu	Tyr	Ala	Val	Lys	Val	Leu
				85					90				95		
Gly	Ala	Xaa	Gly	Ser	Gly	Xaa	Xaa	Ser	Xaa	Leu	Xaa	Xaa	Gly	Xaa	Glu
			100					105					110		
Trp	Ala	Xaa	Asn	Xaa	Xaa	Xaa	Xaa	Val	Xaa	Asn	Xaa	Ser	Leu	Gly	Xaa
	115						120					125			
Pro	Ser	Xaa	Ser	Xaa	Xaa	Xaa	Xaa	Ala	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	130					135					140				
Xaa	Gly	Val	Xaa	Val	Val	Ala	Ala	Xaa	Gly	Asn	Xaa	Gly	Xaa	Xaa	Xaa
145					150					155					160
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Tyr	Pro	Xaa	Xaa	Tyr	Xaa	Xaa	Xaa	Xaa	Ala
				165				170						175	
Val	Gly	Ala	Xaa	Asp	Xaa	Xaa	Asn	Xaa	Xaa	Ala	Ser	Phe	Ser	Xaa	Xaa
			180					185					190		
Gly	Xaa	Xaa	Leu	Asp	Xaa	Xaa	Ala	Pro	Gly	Val	Xaa	Xaa	Gln	Ser	Thr
	195						200						205		
Xaa	Pro	Gly	Xaa	Xaa	Tyr	Xaa	Xaa	Xaa	Asn	Gly	Thr	Ser	Met	Ala	Xaa
	210				215					220					
Pro	His	Val	Ala	Gly	Ala	Ala	Ala	Leu	Xaa	Xaa	Xaa	Lys	Xaa	Xaa	Xaa
225					230					235					240
Trp	Xaa	Xaa	Xaa	Gln	Xaa	Arg	Xaa	Xaa	Leu	Xaa	Asn	Thr	Xaa	Xaa	Xaa
				245					250					255	
Leu	Gly	Xaa	Xaa	Xaa	Xaa	Tyr	Gly	Xaa	Gly	Leu	Xaa	Asn	Xaa	Xaa	Ala
		260						265					270		
Ala	Xaa	Xaa													
	275														

<210> 10
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> assay protein

<400> 10
 Ala Ala Pro Phe
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